05/25/2005

EPA (OPPT, NERL, NRMRL) General Comments: Noack Laboratory "Aerobic Transformation in Soil" protocol

1) According to test, containers will be polypropylene, no glass, no PTFE, screw-tight joints in more than one place, and must be air-tight despite positive-pressure flow-through for a full year. There are concerns that a system like this may leak over time. Could a picture or diagram of the actual system be provided? It is suggested that a preliminary experiment of one month or longer with alcohol spikes be conducted to demonstrate long-term air-tightness by documentation of near 100% recovery.

A picture of the proposed test system for the static system is provided, along with the specifications to order it (vendor and part number).

A method validation will be performed to demonstrate that the test vessels will not leak. TRP will provide an outline of this validation to EPA and the results of the test once it is completed.

2) 40 to 60% water holding capacity is in the steep part of the moisture sorption curve for many soils where small changes in moisture content equate to large changes in moisture potential. Very low potential can kill microbes. Water bubblers commonly hydrate air to within one percent of saturation and not much better based on qualitative observations. Permanent wilting point for plants (15 atm tension) is in excess of 98 or 99% relative humidity according to EPA's calculations. How will it be demonstrated that you will not kill microbes by dessication? One week might be sufficient time to dry a soil to dangerously dry state. Suggest short term moisture-control tests using the test chambers that have been proposed.

A static, rather than flow-through, system will be used. Moisture content of the soil will be checked on a regular basis by weighing and water added as needed.

3) Spike and recovery: There are concerns that recovery of these compounds from soils might not be a constant percentage value as a function of spike concentration. Consider spiking at a few different concentrations or in the range expected for the experiment to address this concern.

During the method validation, a range of concentrations will be spiked into the soil. In the main experiment, a single concentration will be spiked into the sterile spikes at 10 X LOQ. Alternatively, a range of concentrations (for example 1 X LOQ, 10 X LOQ, and 100 X LOQ) could be spiked at t = 0 and then a low, medium, or high spike will be randomly selected for extraction during the course of the study at each of the time points previously defined. This alternative is under discussion within the TRP.

4) Soil Selection for Telomers Biodegradation Testing

Four Soils are Needed to Measure Biodegradation Rates

Mollisol is highly active and represents a large percentage of the land area in the US. A single soil has been selected because the intial test plan will look for potential transformation products, pathways, and the ability of the test system to provide data needed to meet the objectives of the test (the ability of the test to detect polymer degradation). A single soil is recommended in the guidelines (paragraph 23) to determine transformation pathway(s).

Additionally, TRP believes that the transformation rates may be so long (half-lives greater than 500 years) that the transformation rates for different soils would be essentially the same within the experimental error of the analytical methods.

Furthermore, the TRP plan should be viewed as method development so that the laboratory and individual TRP companies can develop collective experience in conducting this type of test. The testing proposed by the TRP, however, does not prevent TRP companies from conducting additional tests on additional test items or soils. It should also be pointed out that the TRP is conducting and planning tests in other media, including aerobic sludge, anaerobic sludge, and sediments.

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1) Anaerobic systems usually are saturated systems. Suggest saturation for the experiment.

The guidelines recommend that the soil is water-saturated by flooding for transformation testing under anaerobic conditions. TRP believes, however, that the soil can be made anaerobic by replacing air with an anaerobic gas mixture that would better simulate conditions in landfills and agricultural soils when sludge is applied, which are where the test items most likely will be disposed. Agricultural soils will drain quickly and will not remain saturated. However, unsaturated anaerobic microsites can occur as a result of aerobic bacteria utilizing the available oxygen. Landfills are designed to minimize or exclude water. These are more appropriate conditions to simulate, TRP believes, than a wetland soil or a rice patty, two situations in which these polymers are unlikely to be found. Additionally, using an unsaturated soil will not require the development and validation of additional extraction and measurement methods so that the aerobic and anaerobic tests can be performed concurrently.

2) Upper 20 cm of mollisols likely will have microbial consortia acclimated to oxic conditions. Anaerobia will have large deleterious effect on these systems. Suggest an organic-rich wetland soil.

Please see previous comment. Wetlands are not obvious places where these test items will be found. Rather, they predominately go to landfills where upland (aerobic) surface and subsurface soils may be used for daily cover. Although these soils are predominantly aerobic, it is well known that they harbor anaerobic microorganisms that will grow and thrive when anaerobic conditions are imposed.

According to Paul and Clark[1] (pg. 17), "It has been determined that the change from aerobic to anaerobic metabolism occurs at O_2 concentrations of less than 1%." "The fact that anaerobic processes such as denitrification and sulfate reduction occur in many soils indicates that anaerobic microsites must occur quite commonly. Further evidence that anaerobic microsites exist can be deduced from the common occurrence of anaerobic bacteria such as clostridia in the upper layers of soil. Several studies have shown that the population of anaerobic bacteria in the upper few centimeters of soil can be as much as 10 times their number at greater depths. Aerobic bacteria play a preparatory role in producing an environment for the anaerobes. Their initial growth within a microsite consumes its stored O_2, thus allowing anaerobes to develop".

3) There is a significant concern for leakage of alcohols out of the systems. To the extent that any leakage at the seals is diffusion driven, as opposed to advection driven, the extremely high $d[O_2]/dx$ gradient from the lab air to microcosm headspace would favor large O_2 leakage into microcosms. Suggest storage of all anaerobic microcosms in a Coy Chamber. If you design the spike and recovery test suggested in comment 1 in the Aerobic Transformation in Soil Comments with N_2 gas in the test chambers, you could analyze the test-chamber for ingrowth of O_2 as well to evaluate integrity of the seals.

The current plan is to store the anaerobic vessels in an anaerobic chamber. However, it will be important to try to establish that the test vessels do not leak so that potential volatile analytes that may form are not lost from the system. A method validation will be performed to demonstrate that the test vessels will not leak.

[1] Paul, E.A., and F.E. Clark. 1989. Soil Microbiology and Biochemistry. Academic Press, Inc. New York. pp. 275.